Serial Processing in the Human Somatosensory System

Although numerous anatomical and electrophysiological findings in animal studies have supported a hierarchical scheme of somatosensory processing, precise activation timings of each cortical area are not known. Therefore we examined the temporal relationship of activities among multiple cortical areas using magnetoencephalography in humans. We found activations in Brodmann’s areas 3b, 4, 1, 5 and the secondary somatosensory cortex region in the right hemisphere following transcutaneous electrical stimulation of the dorsum of the left hand. The mean onset latencies of each cortical activity were 14.4, 14.5, 18.0, 22.4 and 21.7 ms, respectively. The differences of onset latencies among these activations indicated the serial mode of processing both through the postcentral gyrus and through the primary and secondary somatosensory cortices.

Keywords: cortex, human, serial processing, somatosensory system

Introduction

Since Duffy and Burchfiel (1971) and Sakata et al. (1973) showed in unitary recording studies in monkeys that the receptive field (RF) of Brodmann’s area 5 neurons was larger and more complex than the RFs in the primary somatosensory cortex (SI), an increase in complexity of the RF properties in the postcentral gyrus along its rostro-caudal axis has been confirmed by many studies. That is, the complexity of RF characteristics increases from area 3b to areas 1, 2 and 5 (Hyvarinen and Poranen, 1978; Iwamura et al., 1980, 1983, 1994; Sur, 1980; Sur et al., 1985). This increase in complexity is assumed to result from the convergence of multiple inputs to single neurons via serial cortico-cortical connections and, therefore, suggests hierarchical somatosensory processing in the postcentral gyrus (for a review, see Iwamura, 1998). Anatomical studies have demonstrated the serial cortico-cortical connections in these areas (Künzle, 1978; Vogt and Pandya, 1978; Felleman and Van Essen, 1991; Burton and Sinclair, 1996). Anatomical (Vogt and Pandya, 1978; Burton et al., 1995) and physiological (Pons et al., 1987, 1992) studies in monkeys have suggested that such a hierarchical processing also exists between SI and the secondary somatosensory cortex (SII).

In spite of many observations in favor of serial hierarchical processing, there is no study that has precisely investigated the activation timing of multiple cortical areas within the postcentral gyrus and lateral sulcus region both in animals and in humans. If the major flow of signal processing is serially organized, each cortical response should show substantial differences in latency. In the present study, we investigated the temporal relationship among cortical responses to somatosensory stimulation using magnetoencephalography (MEG). Multi-channel MEG has an advantage over single-unit or field potential recordings in that it can easily record activities from multiple cortical areas simultaneously and noninvasively.

Materials and Methods

Thirteen healthy volunteers aged 23–39 (mean 31) years participated in this study. The study was approved in advance by the Ethical Committee of the National Institute for Physiological Sciences and written consent was obtained from all the subjects.

Stimulation

Transcutaneous electrical stimulations were applied to the dorsum of the left hand just on the first metacarpal bone using a conventional bipolar felt tip electrode 0.9 mm in diameter with a distance of 25 mm between the anode and cathode. The electric stimulus was a current constant square wave pulse delivered at a random interval of 1–3 Hz. The stimulus duration was 0.5 ms. The current intensity was three times the sensory threshold (1.0 ± 0.2 mA). Transcutaneous stimulations of the dorsum of the hand at this intensity produce well-defined tactile sensations without painful sensations and evoke clear brain potentials (Inui et al., 2002) and magnetic fields (Inui et al., 2003) due to signals conveyed by A-beta fibers. An electrical stimulation method is suited to the time-locked averaging technique because of the constant activation time.

MEG Recording and Analysis

Somatosensory evoked magnetic fields (SEFs) were recorded using a 57-channel axial-type first-order biomagnetometer (Magnees; Biomagnetic Technologies, San Diego, CA) as described previously (Kakigi et al., 1988, 1992). The probe was centered on the C4 position as based on the International 10/20 System. This position covered the hand area of SI and SII in the hemisphere contralateral to the stimulation (Fig. 1A). The SEFs were recorded with a filter of 0.1–200 Hz at a sampling rate of 2083 Hz. The analysis window was 100 ms before and 100 ms after the stimulus and the prestimulus period was used as the DC baseline. One thousand responses were collected and the average of 800–900 artifact-free responses was used for the analysis.

Source locations and time courses of source activities were determined by a multi-source analysis method, brain electric source analysis (NeuroScan, Mclean, VA) as described previously (Inui et al., 2003). Model adequacy was assessed by examining: (i) percentage variance (Hari et al., 1998); (ii) F ratio (ratio of reduced $\chi^2$ values before and after adding a new source) (Supek and Aine, 1993); and (iii) residual waveforms (that is, difference between the recorded data and the model). Percentage variance ($%V$) is defined as

$$%V = \frac{\sum_{i=1}^{N} (M_i - T_i)^2}{\sum_{i=1}^{N} M_i^2}$$

where $M_i$ are recorded data values, $T_i$ are theoretical (model) values calculated at $N$ measuring points and $%V$ measures the goodness-of-fit of the model comparing the recorded data and the model. In the present study, we used $%V$ for individual data at a selected latency point. We also used $%RV$ (percentage residual variance, 100–$%V$) as the mean value of all the data (0–100 ms). For example, 20$%RV$ indi-
cates that the mean RV value among all sampling points (100 ms at 2085 Hz sampling rate = 208 points) is 20%. \( \chi^2 \) is defined as

\[
\chi^2 = \sum_{i=1}^{N} \left( \frac{M_i - T_i}{\sigma_i} \right)^2
\]

where \( \sigma_i \) are the standard deviations of the noise of each sensor that are calculated from the prestimulus period. In this study, reduced \( \chi^2 \) values were used (\( \chi^2_r = \chi^2/v \), where \( v \) = degrees of freedom = \( N \)-numbers of parameters). The Fratio is defined as

\[
P_{1,2} = \frac{\chi^2_{144}}{\chi^2_{14}}
\]

where \( \chi^2_{144} \) is calculated by a model with \( n \) dipoles and \( \chi^2_{14} \) by a model with \( n + 1 \) dipoles. \( \chi^2_{144} \) and \( \chi^2_{14} \) are distributed according to \( \chi^2 \) distributions of \( N - 5n \) and \( N - 5(n + 1) \) degrees of freedom, respectively. The integral probability of obtaining a Fratio value equal or greater than the obtained value is calculated to evaluate whether a model with a larger number of dipoles represents a statistically significant improvement of the fit over a model with a smaller number of dipoles. When a \( P \) value was <0.05, we considered the new dipole as significant. We continued to add a source to the model until the addition of a dipole did not significantly improve the fit.

Sources were superimposed on the individual magnetic resonance images (MRI; 150XT 1.5 T; Shimadzu, Kyoto, Japan). The source location was expressed using an MEG head-based coordinate system. The origin was the midpoint between the pre-auricular points. The \( z \)-axis indicated the coronal plane with a positive value in the anterior direction, the \( y \)-axis indicated the mid-sagittal plane with a positive value toward the right pre-auricular point and the \( z \)-axis indicated the transverse plane pre-auricular to the \( x \)-\( y \) plane with a positive value toward the upper side.

A one-way analysis of variance (ANOVA) followed by Bonferroni/Dunn’s post hoc test was used for statistical comparisons of the latency among each cortical activity. The statistical significance of the source location was assessed by a discriminant analysis using \( x \), \( y \), and \( z \) coordinates as variables. \( P \) values < 0.05 were considered to be significant.

**Results**

Since recorded magnetic fields in each coil were a summation of those from temporally overlapping multiple source activities, a multiple source analysis method was used to differentiate each source activity. The procedure and results of the analysis in a representative case are shown in Figure 1. In all cases, well-known 20 and 30 ms responses were major components of the recorded magnetic fields and we started the analysis with these activities. The recorded magnetic fields at 20 and 29 ms in Figure 1 showed similar typical one-dipole patterns in opposite directions (Fig. 1Ba) and the best source location to explain the field pattern of this latency was estimated to be in the posterior bank of the central sulcus, corresponding to area 3b. The first trace in Figure 1C shows the time course of the source strength of this source. This source could explain 52.8% of the recorded data (mean RV was 47.2%). In the next step, magnetic fields due to the area 3b source were subtracted from the recorded magnetic fields. Therefore, the subtracted magnetic fields (Fig. 1Bb) were due to activities from sources other than the area 3b source. In the subtracted waveform (Fig. 1Bb), 25 and 35 ms magnetic fields showed similar and clear dipole patterns in opposite directions. As shown in the isocontour map (Fig. 1Bb), the major contributor to these magnetic fields was located slightly posterior and superior to the area 3b source. The superimposition of this source on MRI showed that it was located in the anterior crown of the postcentral gyrus, suggesting that the source was in area 1. After the location and orientation of the area 3b and area 1 sources were slightly adjusted to provide the best fit of the recorded data with these two sources, the mean RV value of the whole analysis period was 20.7%. At the peak latency of the area 1 activities (25 ms), \%V was markedly increased from 2.3 to 95.5% by adding the area 1 source.

In the next step, magnetic fields due to these two sources were subtracted from the recorded data. The subtracted magnetic fields were those that remained to be explained. In the subtracted waveform (Fig. 1Bc), weak deflections around 20 and 30 ms showed a relatively clear field pattern and the best source to explain the waveform was estimated to be in the posterior crown of the precentral gyrus, corresponding to area 4. By adding the area 4 source, \%V (at 21 ms) was increased from 94.7 to 99.5% (\( F = 12.6 \), \( P < 0.0001 \)). In the subtracted waveform (Fig. 1Bd), which could not be explained by these three sources, isocontour maps at a latency of 60–100 ms constantly showed a single dipole pattern. This source activity was estimated to occur in the upper bank of the Sylvian fissure, corresponding to SII plus adjoining areas (late SII+ source). We referred to this area as SII+ because human MT plus adjoining areas is referred to as MT+. At the peak latency of the late SII+ activity (95 ms), \%V was increased from 73.7 to 96.9% (\( F = 8.7 \), \( P < 0.001 \)). At this step, the mean RV was 8.8%. The waveform of the residual magnetic fields (Fig. 1Bc) had several deflections at around 30, 40 and 55 ms, but isocontour maps at these latencies showed a two-dipole pattern. One source was estimated in a region posterior and medial to the area 1 source, around the intraparietal sulcus (posterior parietal cortex, PPC source) and the other near the late SII+ source (early SII+ source: \( F = 3.1 \), \( P = 0.028 \) at 29 ms; \( F = 8.5 \), \( P < 0.001 \) at 39 ms; \( F = 9.8 \), \( P < 0.0001 \) at 55 ms). After fitting these six sources, the mean RV (0–100 ms) was 0.9% and any additional source did not significantly improve the fit. Figure 1D shows the location and orientation of each source. Figure 1C shows the time courses of each source strength and these were used for the analysis of the latency of each activity. In this case, the time course of each source activity showed a similar triphasic pattern.

Similar procedures were applied to data obtained for the remaining 12 subjects. After confirming the precise location of each source in individual MR images, activations in area 3b were identified in all 13 subjects. Sources in area 1, area 4 and PPC were found in 12, nine and eight subjects, respectively. Because two SII+ sources showed an apparently different time course of activity, we analyzed these two activities separately. Both early and late SII+ sources were identified in 10 subjects. In general, all source activities except for the late SII+ activity had two major deflections in opposite directions with a 10 ms interval. Figure 2 shows the time course of each cortical activity of all subjects. The mean onset and peak latencies are shown in Table 1. The onset latency of the area 3b source (14.4 ms) was the shortest but was not different from that of the area 4 source (14.5 ms). The onset latency of the area 3b source was significantly shorter than that of the area 1 source (18.0 ms) and, in turn, the onset latency of the area 1 source was significantly longer than that of the area 3b or area 4 source.
The area 4 source was located more anterior (6.9 mm) than the area 3b source but its overall location was not different ($P = 0.13$, discriminant analysis). The area 1 source was located more medial (9 mm) and posterior (7.2 mm) than the area 3b source ($P = 0.0002$) and, in turn, the PPC source was located more medial (9.2 mm) and posterior (10.9 mm) than the area 1.

Figure 1. Procedures and results of the data analysis. (A) Sensor layout. (B) Superimposed waveforms recorded from 37 channels (a), residual magnetic fields obtained by a subtraction of those due to one (b), two (c), three (d), four (e) and six (f) sources determined from the recorded data. Isocontour maps at the peak latency of a selected deflection (vertical bars) are shown on the right side of each trace. (C) Time course of each source strength. (D) Schematic drawings of the location and orientation of each source. Bars indicate the direction of upward deflections of the corresponding waveform in C. (E) Superimposition of sources on a subject’s brain surface image. White circles in A and isocontour maps indicate the position of the sensor (channel 3) that is just on the central sulcus. SII+, secondary somatosensory cortex plus adjoining areas; PPC, posterior parietal cortex; RV, residual variance.
The source for the first activities peaking at 21 and 30 ms was located in the posterior bank of the central sulcus, corresponding to area 3b. This finding was consistent with previous somatosensory evoked potential (SEP) and SEF studies demonstrating that the earliest cortical responses to median nerve stimulation originate from area 3b in humans (Wood et al., 1985; Allison et al., 1989) and monkeys (McCarthy et al., 1991). The second activity in SI peaking at 25 and 34 ms was located more medial (9 mm), superior (12.7 mm) and posterior (7.2 mm) than the area 3b source, around the anterior crown of the postcentral gyrus, suggesting that this activity originated from area 1. Using intracranial SEP recordings in humans (Allison et al., 1989) and monkeys (Allison et al., 1991), it has been demonstrated that a 25/35 ms activity is produced by a radially oriented generator located in the anterior crown of the postcentral gyrus in area 1, in a region ~10 mm medial to the region of 3b potentials peaking at 20 and 30 ms. Both the source location and the time course of the activity are consistent with the present results. The onset latency of the area 1 activity was longer by 3.6 ms than that of the area 3b activity. Therefore, our finding was congruent with the serial activations in areas 3b and 1, which is supported by the anatomical finding in monkeys that area 3 projects predominantly into area 1 (Vogt and Pandya, 1978) and an electrophysiological study in monkeys that showed that ablations of area 3 immediately abolished cutaneous responsivity in area 1 (Garraghty et al., 1990a). The mean distance between sources in areas 3b and 1 in this study was ~17 mm. Given that the synaptic delay is 1 ms, the conduction velocity of this projection is 6.5 m/s (17 mm/2.6 ms). Since there are sparse projections from the thalamus to area 1 (Jones and Powell, 1970; Nelson and Kaas, 1981), there might be earlier activities in area 1 that were driven by this direct pathway. However, we could not find earlier activities in this region. As in previous MEG studies in humans, the present study did not detect activities from area 2. This seems largely due to the fact that area 2 is located in the crown of the postcentral gyrus. MEG cannot detect activities from radial dipoles such as those in the crown.
of gyri. The area 1 activities in this study probably originated from fissural parts of the postcentral gyrus, activities from which would create tangential magnetic components to some extent. However, there remains a possibility that our area 1 activities actually contained activities from area 2.

The third source in the postcentral gyrus was in its caudal-most part around the intraparietal sulcus, probably corresponding to area 5. Although a few previous MEG studies reported activations in this area following somatosensory stimulation at a latency of 50-100 ms (Forss et al., 1994; Hoshiyama et al., 1997), this is the first report to demonstrate the early activities in this region. The onset latency of this activity was significantly longer (4.4 ms) than that of area 1. Since area 5 receives main inputs from areas 1 and 2 (Pons and Kaas, 1986) and, in turn, area 2 receives inputs mainly from area 1 in monkeys (Vogt and Pandya, 1978), the responsiveness to cutaneous stimuli of area 5 appears to depend on direct relays from area 1 and area 2 and on a serial relay from area 1 to area 2 and then to area 5.

Regarding the two sources in the lateral sulcus region, we consider these two activities to come from different groups of neurons in this area, since they showed a different source orientation and time course. This notion is consistent with the fact that this region has been divided into at least two parts based on anatomical (Burton et al., 1995) and electrophysiological (Krubitzer et al., 1995) findings in monkeys and cortical surface (Mima et al., 1997) and intracortical (Frot and Mauguire, 1999; Barba et al., 2002) SEP findings in humans. We considered that our two sources in the lateral sulcus region probably corresponded to SII and PV (parietal ventral area), the regions that have been intensively studied by Krubitzer and colleagues in both animals and humans. PV is a somatosensory area first reported for squirrels (Krubitzer et al., 1986; Krubitzer and Kaas, 1987). Electrophysiological studies of the SII region in primates (Krubitzer and Kaas, 1990; Krubitzer et al., 1995; Qi et al., 2002) demonstrated that the region historically referred to as SII is actually composed of at least two separate areas, SII and PV, each of which contains a complete representation of the body surface. In addition, distinct cortico-cortical (Disbrow et al., 2003) and thalamocortical (Krubitzer and Kaas, 1992; Disbrow et al., 2002; Qi et al., 2002) connections of SII and PV have been studied. These studies have established SII, as well as PV, as one of multiple somatosensory areas of the lateral sulcus in primates. Recently, evidence for SII and PV in humans has been provided in a functional MRI study (Disbrow et al., 2000).

The peak latencies of the major two components of the early SII+ source were 30 and 40 ms and those of the late SII+ source were 56 and 90 ms, which were very similar to two separable components in the SII region following median nerve stimulation, N30op and N60p, in intracortical SEP studies in humans (Frot and Mauguire, 1999; Barba et al., 2002). The onset latency of the early SII+ source was significantly longer than that of the area 3b source (7.5 ms), suggesting a serial mode of processing in SII and SI+. Given that signals reach the SI region via area 3b with one synaptic transmission, the estimated conduction velocity of this cortico-cortical connection is 4.3 m/s (2.7 cm/6.3 ms). Anatomical (Jones and Powell, 1970; Friedman et al., 1980) and electrophysiological (Pons et al., 1987, 1992; Garraghty et al., 1990b) studies in monkeys support a serial mode of processing through SI and SII. Findings in previous MEG studies in humans are also consistent with the present results, showing that the somatosensory region in the sylvian fissure is serially activated from SI (Hari et al., 1984; Elbert et al., 1995; Disbrow et al., 2001). However, recent studies in marmosets provided evidence for hierarchical equivalence of SI and SII for tactile processing (Zhang et al., 2001a,b). Findings in an MEG study (Karhu and Tesche, 1999) were also in favor of parallel processing in SI and SII showing simultaneous activations in these areas. Therefore, there remains a possibility that we missed weak activities in the SII region prior to those in the present study.

The onset latency of the area 4 source was not different from that of the area 3b source, indicating that the initial activity of this source at least was independent of activities in SI, which was consistent with anatomical findings that projections from area 3b to area 4 were absent or very weak (Vogt and Pandya, 1978; Darian-Smith et al., 1993; Burton and Fabri, 1995). We considered that area 4 was driven by thalamic inputs (Ghosh et al., 1987; Stepniewska et al., 2003), although later activities of this source might come from area 1 or 2 (Ghosh et al., 1987; Darian-Smith et al., 1993; Burton and Fabri, 1995). Because of the proximity of areas 3a and 4, it is possible that our area 4 activity was actually from area 3a. However, area 3a receives main inputs from deep tissues (Icath et al., 1976) and our cutaneous stimulation method seems not to be effective at activating deep tissues. In addition, the area 4 source was located more superior (4.4 mm) than the area 3b source in the present study, which also did not support this possibility.

The present results showed that most of the source activities had polarity reversals after 10 ms, that probably corresponded to the surface positive-negative sequence of potentials recorded from the cortex of experimental animals. These positive-negative sequences have been recorded from somatosensory, visual and auditory cortex (for reviews, see Schlag, 1973; Mitzdorf, 1985) and are often called the primary evoked response (Towe, 1966). In general, the primary positivity is thought to reflect the initial depolarization of pyramidal cells and proximal apical dendrites, whereas the primary negativity is thought to be a surface reflection of the later depolarization of the distal apical dendrites (Landau, 1967; Schlag, 1973; Wood and Allison, 1981). Under this condition, two successive dipoles with opposite directions occur. By the use of current source density analyses, many animal studies have found, regardless of species or sensory modalities, a combination of an early sink near cortical layer 4 and a corresponding source in layer 5, and a 5–10 ms later polarity reversed dipole with a sink in layer 2/3 and a source in layer 1 (e.g. Kossut and Singer, 1991; Steinschneider et al., 1992; Peterson et al., 1995; Pearce et al., 2000).

Although there remains a possibility that all these activations came directly from the thalamus and the different response latency was due to the different conduction velocity of the pathway between the thalamus and each cortical area, the present results together with many previous findings suggest that the main flow of somatosensory processing is serially organized in these areas. In addition, it has been shown in animals that the latency from the thalamus to a cortical cell is remarkably constant across multiple cortical areas, irrespective of the variability of traveling distance (Salami et al., 2003). The present MEG study clearly separated activities in multiple cortical areas and could show the temporal relationship among them. Although it is obvious that single-unit recording studies in conscious animals are important for understanding somato-
sensory processing, MEG can serve as a noninvasive method to study the timing of arrival of signals to multiple cortical areas in humans.

**Notes**
Address correspondence to Koji Inui, Department of Integrative Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki, 444-8585, Japan. Email:inui@nips.ac.jp.

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